MUCORALES FROM THE "BARADLA" CAVE IN AGGTELEK
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by

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The present paper deals with fungi of the cultivated *Mucorales* series isolated from the „Baradla" stalactit cave, in north-eastern Hungary, during a mycological exploration of this region.

These investigations have resulted so far in the isolation of 17 strains, representing, with a total of 12 species, 5 genera of the *Mucoraceae* and 1 genus of the *Cunninghamellaceae*.

Material and Method

Soil sample and bat guano served as sources of the fungi. The samples were always taken with sterilized instruments. The sampling flasks with their cotton plugs, as well as the packed scoops were sterilized with dry heat for 2 hours at a temperature of 160 °C.

The soil samples were taken about 5 – 10 cm from under the ground level, while the top layer was thrown away.

The following culture methods were applied:

1. The classical method for growing soil microorganisms, i.e. the preparation of a dilution series, and plated over the surface of Czapek-Dox, Sabouraud-glucose, malt and potato-dextrose agar. K-penicillin (40 U/1000 ml and streptomycin (80 U/1000 ml) were added to the culture medium in order to suppress the bacterium flora. In this case, however other microscopic fungi, present in large numbers in the soil, were also growing on the whole surface of the culture medium. Therefore the technique was modified.

2. A small amount of the sample (as big as a hazel-nut) was placed in the middle of the surface of the agar plates with the above composition. Although the number of germs was much higher now than in the case of the above method, the number of fungal colonies which actually developed was lower. Spores and conidia, getting into contact with the culture medium, were developing primarily. Since the colonies of *Mucorales* grow larger than those of other

microscopic fungi after the same culture period, we were able to ensure them spacial advantage.

The result obtained (1st method 4 species, 4 strains, 2nd method 2 species, 2 strains)* proved to be unsatisfactory, the T o K a V a (T o m a – K a r l i n g – V a n b r e u s e g h e m) bait method was tested. This method has been successfully applied for growing keratin-decomposing microorganisms. Since the *Mucorales* may be primarily considered as saprophytic organisms, baits containing organic substances were used.

3. An approximately 1/2 cm thick layer of the sample was put in Petri dishes, with sterilized rabbit manure and lumps of bread placed on its surface. The bait was sterilized in an autoclave for 30 minutes, at an overpressure of 1 atm. and a temperature of 121°C. Proper humidity was secured with sterile water.

4. A small sample was placed in the middle of sterile filterpaper discs put in Petri dishes. In order to supply inorganic salts for the development of the fungi, W i n o g r a d s k y's standard solution was used for water.

In every case the incubation of the material was carried out in a thermostat adjusted to 28°C. Pure cultures were obtained from fully developed colonies on malt agar.

Results

Familia	Genus	Species	Number of strains	Method
Mucoraceae	Mucor	plumbeus	1	dilution
	Mucor	racemosus	1	dilution
	Circinella	simplex	1	culture medium surface
	Actinomucor	elegans	3	bread, rabbit manure bait
	Rhizopus	arrhizus	2	dilution, rabbit manure bait
	Rhizopus	Delemar	1	dilution
	Rhizopus	nigricans	1	filter-paper
	Absidia	Blakesleana	1	rabbit manure
	Absidia	glauca	1	culture medium surface
	Absidia	corymbifera	2	filter-paper, bread bait
Cunninghamellaceae	Cunninghamella	echinulata	1	rabbit manure bait
	Cunninghamella	elegans	2	rabbit manure bait

Taking the number of isolated strains as a basis, the use of rabbit manure bait proved to be the most efficient technique with 7 strains. It is followed by the first method with 4 strains, and subsequently by the 3 others with 2 strains each. However, this order of succession does not reflect correctly the efficiency of the methods. The dilution method was initially set up for growing other microscopic fungi. Of course, any developing *Mucorales* were also isolat-

* Identical species obtained from 5 parallel of samples originating from one and the same collection are regarded as a single strain.

ed. The strains isolated from several hundreds of Petri dishes showed a various fungi to *Mucorales* ratio of 217:4. We were unable to set up comparable proportions for the other methods, since these were specially designed for culturing of *Mucoraceae* and none but the latter were isolated. However, an approximate idea can still be formed, considering for instance that the rabbit manure bait method was employed with 13 samples with 2 parallels each, i. e. with 26 Petri dishes. The number of the isolated strains was 7, respecting 5 species. The bread bait method was applied for 5 samples with only one Petri dish for each, and even so we were able to isolate 2 species.

Mucor plumbeus Bonorden

The species developed on 1 out of 5 parallel plates from the soil sample collected from beside the dripstone formation "Petőfi kútja"* (Fountain of Petőfi) and plated in a 10 000-fold dilution on Sabouraud-glucose-agar.

Due to its characteristic morphological properties it could be indentified beyond any doubt.

The greyish-brown colony is 2 – 5 mm high. Upon ageing the sporangiophores are branched. The sporangia are brown and have a spiny wall, 40 – 80 μ in size. The columella is most variable in size and shape. Oval when young, it becomes cylindrical or clubshaped later on, with characteristic protuberances and spines of variable number and shape, 20 – 50 × 15 – 30 μ in size.

The sporangiospores are ligth brown, rough, spherical or ovoid and 4,8 × 9,6 μ in size.

Mucor racemosus Fresenius

The species was grown on malt culture medium from a 100 000-fold dilution of the soil sample collected from the side wall beyond the "Petőfi kútja" (Fountain of Petőfi) at an approximate height of 1,20 m.

The colony is of brown colour and of a height ranging from 2 to 100 mm. The sporangiophores are strongly branched. The sporangia are 20 – 60 μ in size. The columellae are spherical, oval or slightly pyriform and 10 – 40 × 6,4 – 20 μ in size. The sporangiospores are shperical or slightly oval and 4 – 8 × 4 – 10 μ in size. Gemmae can be found in large numbers in the vegetative hyphae, reminding of a bead-string, but also occur in the sporangiophores and the columellae.

Circinella simplex van Tieghem

The species developed on Sabouraud-glucose agar from bat guano accumulated under the resting-place of bats at the entrance of the "Vámpirok terme" (Vampire Hall).

* The names in quotation marks will stand forthwith for the names of dripstone (stalactite/stalagmite) or cave sectors.

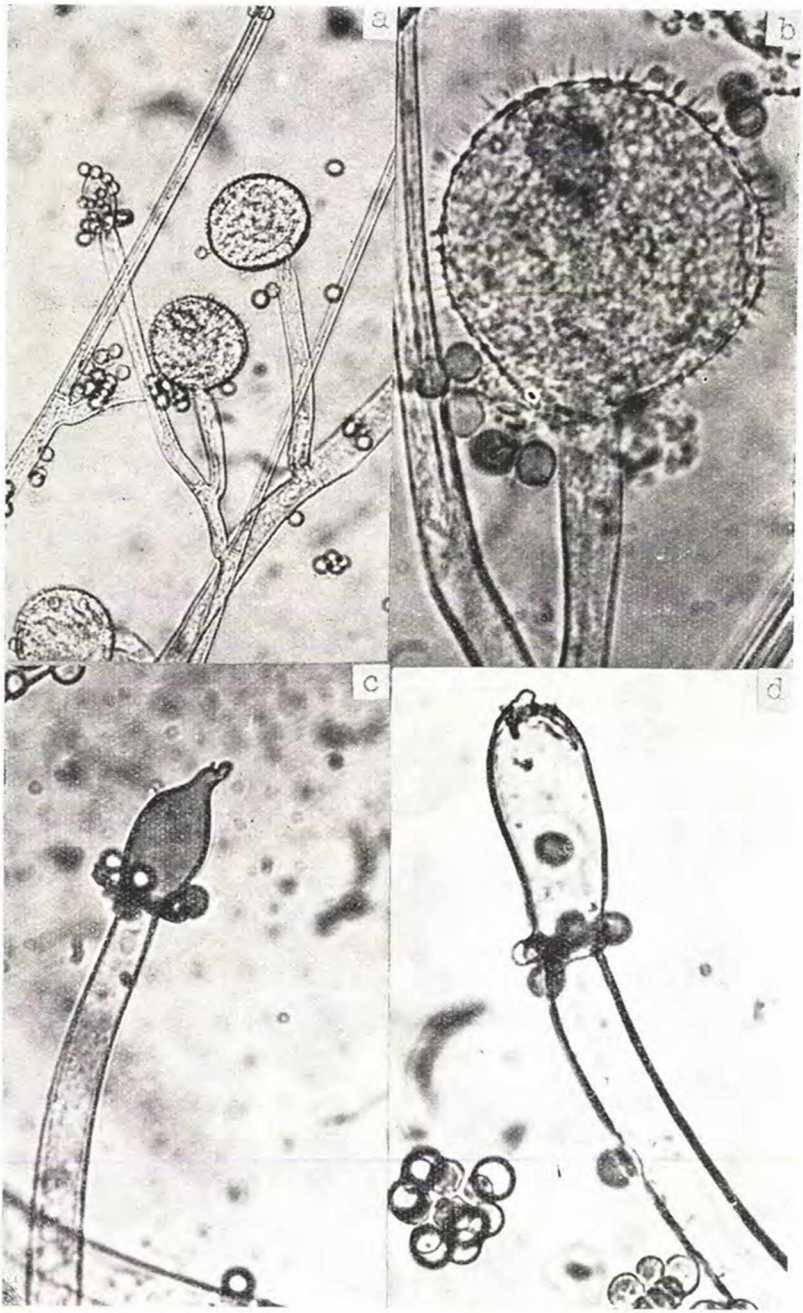

Fig. 1. *Mucor plumbeus*. a: branched sporangiophore (250×), b: young spinescent sporangium (1000×), c–d: columellae with terminal spines (1000×)

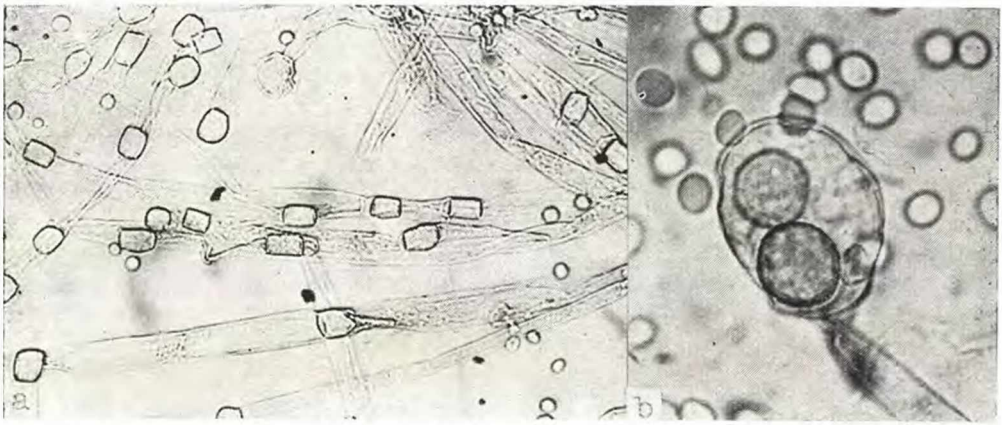

Fig. 2. *Mucor racemosus*. a: chain-like developed chlamydospores (250×), b: columella with chlamydospores (750×)

The colony is hardly 2 mm heigh and of a light brown colour. The sporangiophores are branched, the side branches are short and bending down towards the main axis. The sporangia have an easily bursting wall, 20 – 40 μ in size. The columellae are small, spherical or slightly conical, smooth and creased in old cultures. Most of the sporangiospores are spherical, while some of them are of oval or irregular, 3 – 5,6 × 3,2 – 4,8 μ.

Actinomucor elegans (Eidam) Benjamin et Hesseltine

This species was isolated from three distant sampling sites. It developed from lake-bottom mud from beside the "Nádor-oszlop" (Palatine Column) – on bread bait, from loamy soil from beside „Petőfi koporsója" (Petőfi's Coffin) and from bat guano collected in the middle of the "Vámpirok terme" (Vampire Hall) – on rabbit manure bait.

Two of the three strains are of a light drab colour mixed with white of almost the same shade. One strain is of a drabbish grey. As for other properties there are no remarkable differences between the three strains. The colony is about 10 mm heigh. The sporangiophores are of most unequal lengths and

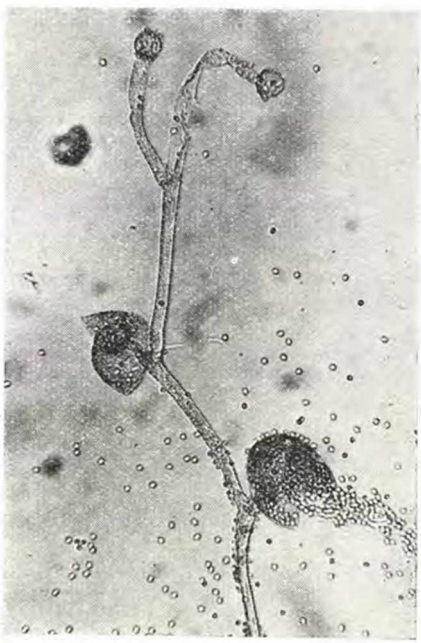

Fig. 3. *Circinella simplex* · sporangiophore (250×)

branchings. Always thinner than the main branch, the lateral branches are either verticillate or racemose on account of secondary branching. At the starting point of the lateral branches the main branch often gets broader and inflated. The sporangia are spherical, their wall is strongly spiny, seldom smooth and bursting. The terminal primary sporangium is always larger than the secondary sporangia on the lateral branches. Primary sporangia are $80-100$ μ, the secondary ones $20-40$ μ. Depending on the size of the sporangia, that of the columellae varies between $16-60\times12-40$ μ. The larger ones are spherical, oval or pyriform, while the smaller ones are shperical, globose, dorsiventrally flattened or conical. The sporangiospores are spherical, $5{,}6-8$ μ, smooth or rough.

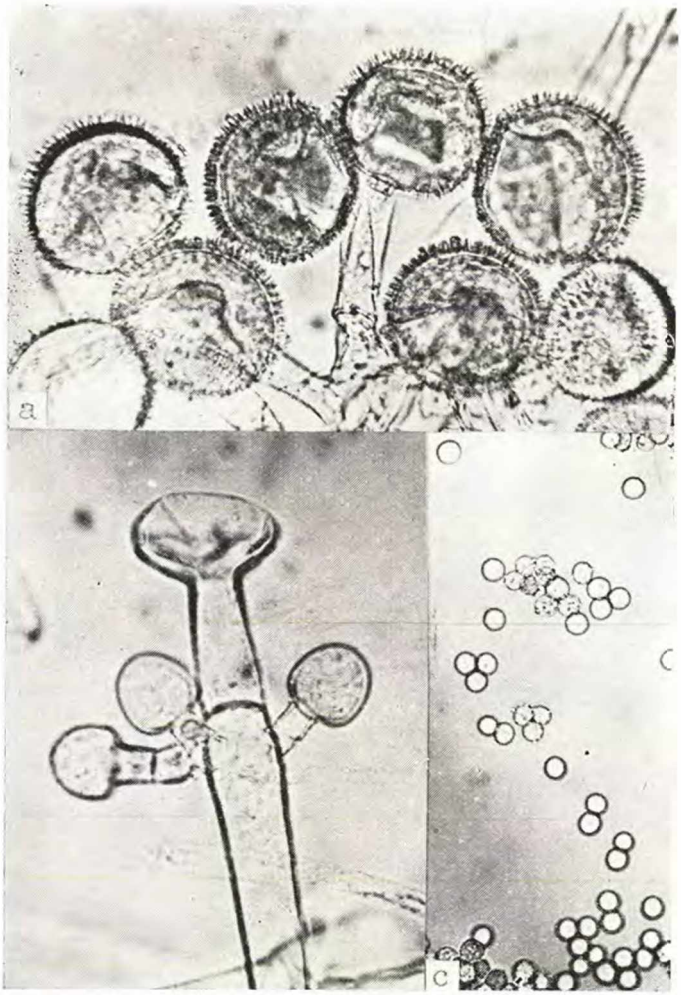

Fig. 4. *Actinomucor elegans*. a: short-stalked bunch of sporangia (250×), b: terminal part of a sporangiophore with columellae (500 ×), c: spores with smooth and rough walls (300×)

Rhizopus arrhizus Fischer

The species was isolated in two cases. It developed on rabbit manure bait from the soil collected on the left hand side of bridge No. 11, and on malt medium from a 10 000-fold dilution of bat guano soil collected from the middle of "Vámpirok terme" (Vampire Hall).

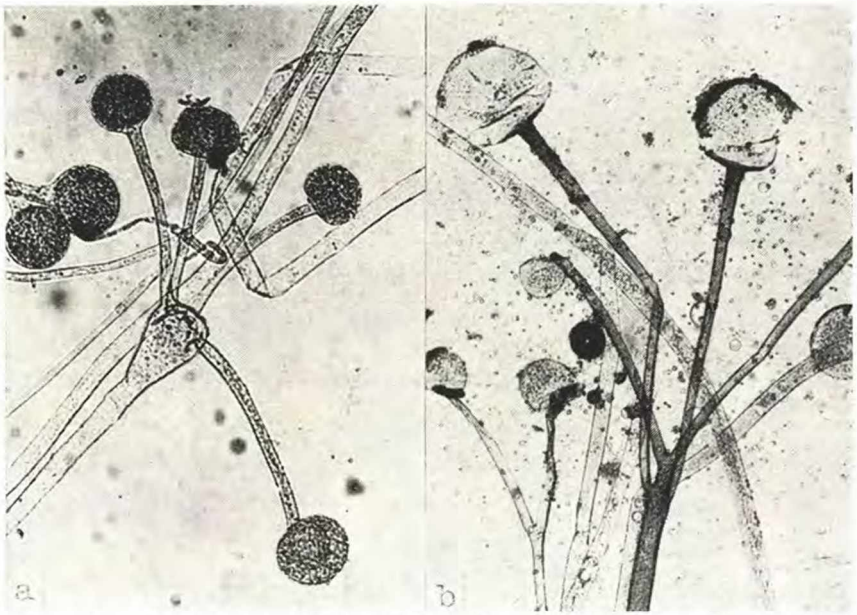

Fig. 5. a: *Rhizopus arrhizus* (100×), b: *Rhizopus Delemar* (100×)

The colony is of dark greyish brown colour and 10 – 15 mm high. There are but few rhizoids to be found in the colony itself, they can be observed mainly on the wall of the dish, and they are poorly developed. Stolones and sporangiophores get brown in an old culture. Sporangiophores occur seldom alone and mostly in clusters of 2 to 6. The starting point is swollen (bulbous). The sporangia are 80 – 200 μ in size. The columellae are hemispherical, spherical or oval and 40 – 110 × 30 – 100 μ in size. The spores are subglobose, 5,6 – 8 (10) μ.

Rhizopus Delemar (Boidin) Wehmer et Hanzawa

This species was grown with the dilution method on potato-dextrose agar from guano accumulated under the resting place of bats in "Vámpirok terme" (Vampire Hall) – at the border of the big hall.

The colony is greyish brown and 10 – 25 mm high. The species is closely related to *Rh. arrhizus*. The rhizoids are few in number and poorly developed. Stolones and sporangiophores become also brown, but not as intensively as those of *Rh. arrhizus*. The sporangiophores stand in clusters of 1 to 4, but without any bulbus at the starting point. They are 400 – 1000 μ in length. The

sporangia are 80 – 180 μ. The columellae are spherical or oval and 20 – 120 × 16 – 100 μ. The sporangiospores are subglobose or oval or angular with obtuse edges, 4 – 11,2 μ in size.

Rhizopus nigricans Ehrenberg

The colony of this species developed with the filter-paper method from dried bat guano gathered in the „Vámpirok terme" (Vampire Hall).

The colony is of a dark grey, almost black colour, with many well developed rhizoids, where the stolones and the sporangiophores in clusters of 2 – 7 start from. There are also solitary sporangiophores to be observed in young cultures that are at the initial stage of development. The sporangiophores are of 1 – 2 mm length. The sporangia are 80 – 250 μ in size. The columellae are hemispherical, spherical and seldom oval, with a broad apophysis. The sporangiospores are subglobose or oval, with obtuse edges and striated surface; they are 7 – 10 × 10 – 16 μ. The sporangiophores and the rhizoids get brown, while the stolones remain hyaline even in old cultures.

Absidia Blakesleana Lendner

The colonies of this species developed on rabbit manure bait from a soil sample collected at the entrance of the passage named "Retekág" (Radish-Branch).

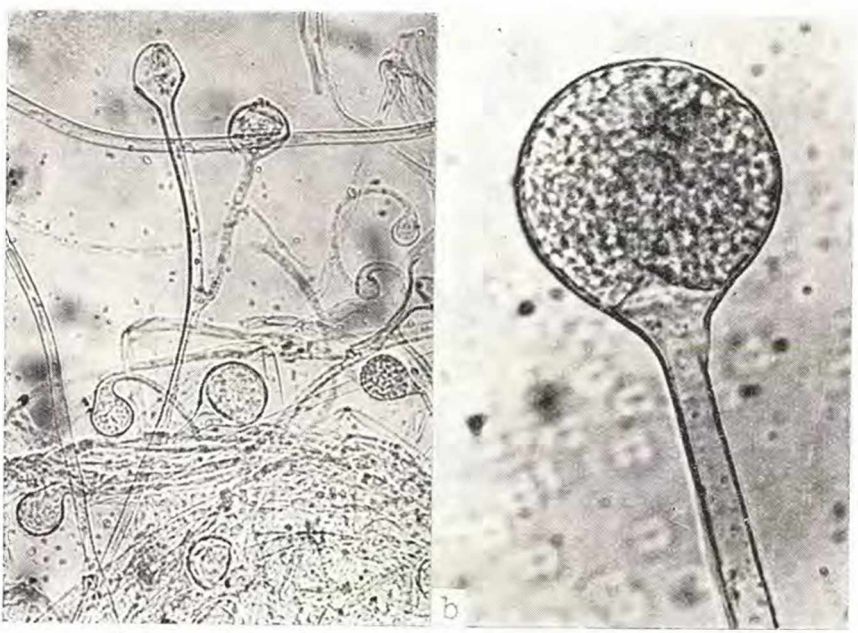

Fig. 6. *Absidia Blakesleana*. a: general view of conidiophores with characteristic bowing sporangia (300×), b: mature sporangium (900×)

The colony is of a light drab colour. Contrary to literary data, it is 10–15 mm high. The sporangiophores may be straight and simple, or wear lateral branches bending towards the main branch, characteristic of the species. Its appearance is therefore like that of the genus *Circinella*. The terminal sporangia are 25–40 μ, the lateral ones 10–20 μ in diameter. The columellae are hemispherical, ellispsoidal or pyriform; a collar remaining from the bursting sporangium wall can be frequently observed. The spores are oval or, less frequently, spherical; their size is variable 5–6×4–5 μ; the extreme values measured were 2,4×2,4 and 8×4 μ.

Absidia glauca Hagem

The colony of this species developed from fresh bat guano collected at the entrance of "Vámpirok terme" (Vampire Hall) on the surface of a Sabouraud-glucose plate. The strain formed a zygospore and was of a brown colour. Relying upon literary data the only accepted homothallic species *A. septata* van Tieghem could not be taken in account, since in the case of our strain the two suspensors do not originate from a single branch, but from a separate

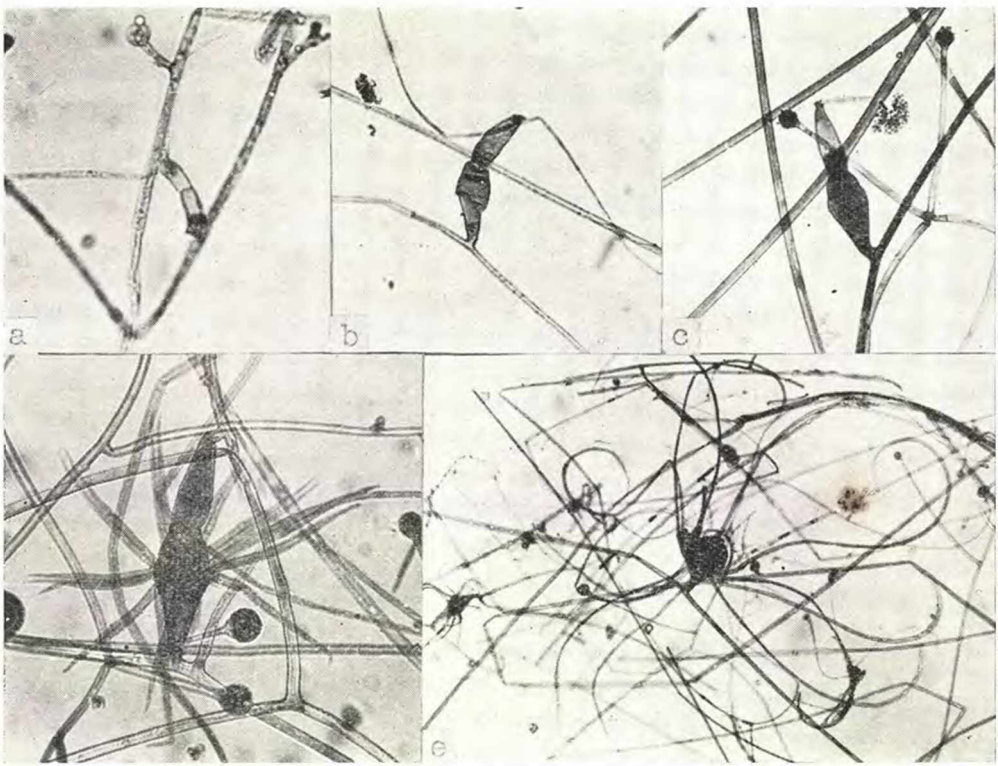

Fig. 7. *Absidia glauca*. a–b–c–d: four phases in zygospore formation (100×), e: mature zygospore (35×)

hypha each. In order to identify our strain we had to demonstrate its heterothallic character. We actually succeeded in isolating a + and a − mating type.

The fully developed colony is brown and 15 – 20 mm high. The characteristic blue-green colour can be readily observed on malt culture medium as long as the sporangia are unripe, i.e. their brown colour does not prevail. Starting from the stolon arch, the sporangiophores inclusters of 1 – 4 are 200 – 800 μ in length, some of them are branched. The sporangia are 25 – 60 μ in size with a septum below the apophysis. The columellae are hemispherical, some of them slightly tapered, 20 – 50 μ. The sporangiospores are smooth, 2,4 – 3,5 μ. The fully developed zygospore is black and 100 – 150 μ; originating from the suspensor, a large number (10 – 20) of long incurved appendices surround it.

Absidia corymbifera (Cohn) Sacc. et Trotter

The species was isolated at two occasions from bat guano collected at the entrance of "Vámpirok terme" (Vampire Hall). It first appeared on filter-paper and for the second time on bread bait.

The colony is greyish brown and 15 – 20 mm high. The sporangiophores are of a monopodial or a sympodial type of branching, the stolons originate also from the sporangiophores. The sporangium is of highly variable size with its diameter ranging from 10 to 70 μ. The columella is hemispherical and slightly conical; in most cases there is a septum below the apophysis. The sporangiospores are partly spherical and partly of a short oval shape, 2 – 3 × 3 – 4 μ.

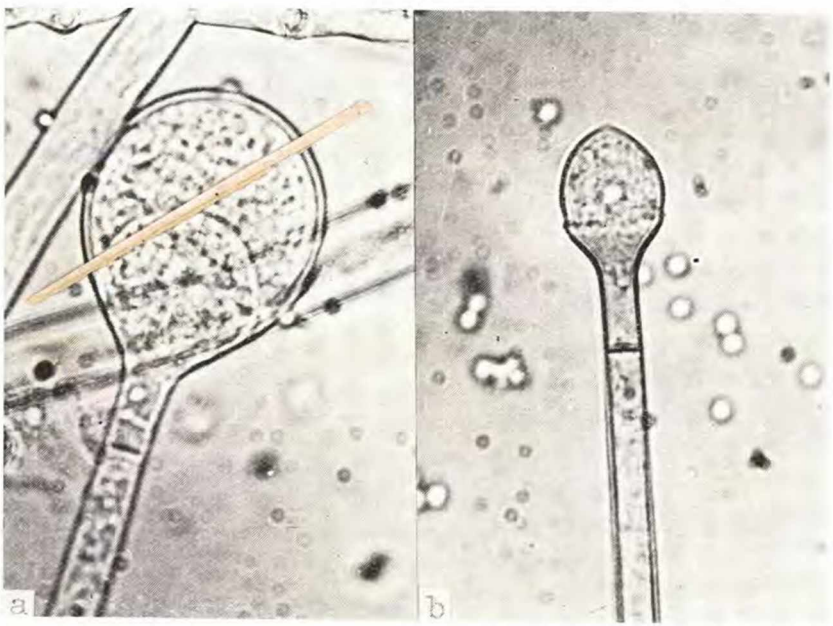

Fig. 8. *Absidia corymbifera*. a: sporangium (600×), b: columella with small collar-residue (600×)

Cunninghamella echinulata (Matruchot) Thaxter

The colony of this species developed on rabbit manure bait from a soil sample collected from beside "Petőfi koporsója" (Petőfi's Coffin). The colony is brown and about 20 mm high. The conidiophores have verticillate or sympodially arranged lateral branches. The terminal vesicle is always bigger than the lateral ones. Their surface is covered with small sterigma bearing the conidia.

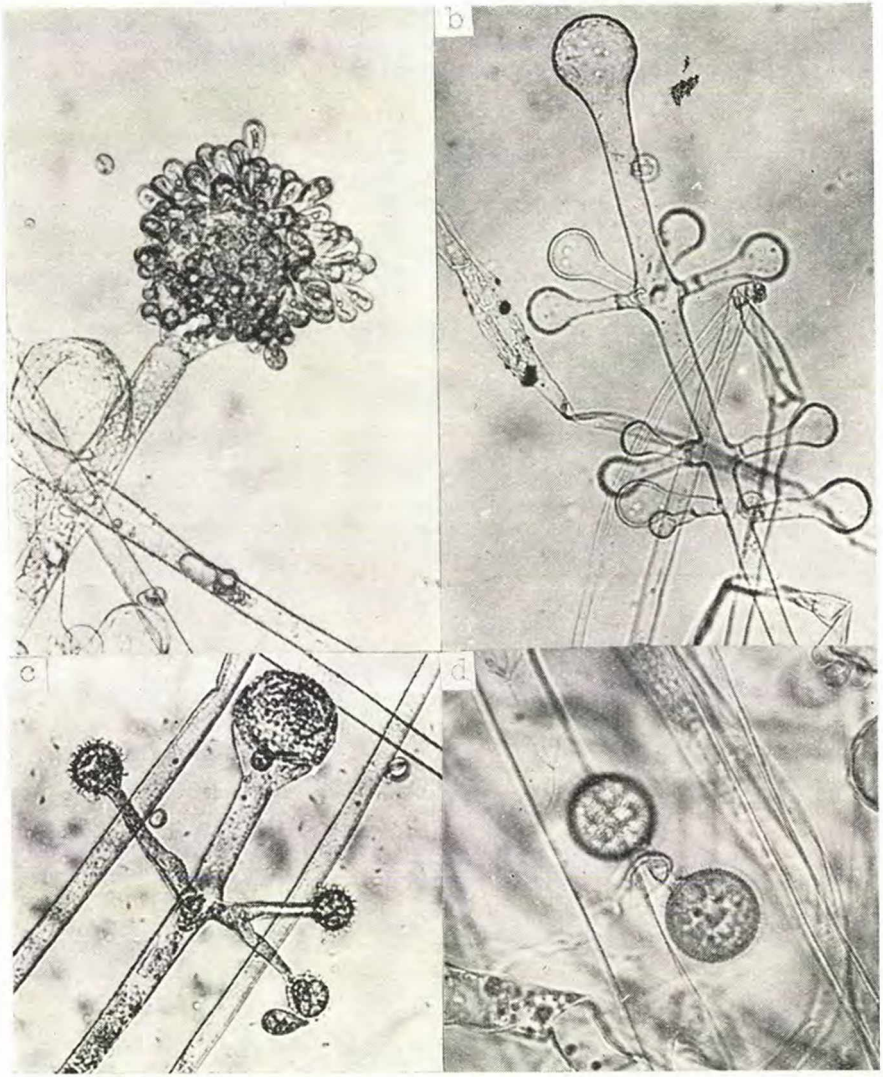

Fig. 9. *Cunninghamella echinulata*. a: primer terminal head of a conidiophore covered with oval conidia (600×), b: manifold branched conidiophore (600×), c: simple branched conidiophore (600×), d: secondary head with two big spinous conidia (800×)

The terminal vesicle is 30 – 80 μ, the lateral ones 16 – 30 μ. There are two different kinds of conidia. Some of them are oval, 6 – 8 × 12 – 16 μ, echinulate. They are formed in large numbers on the terminal or lateral vesicles. Others are round, 10 – 25 μ, covered with 1 – 2 μ spines; they are few in number and are formed always on the lateral vesicles.

Cunninghamella elegans Lendner

The species was grown on rabbit manure bait from lake-bottom mud collected from beside the "Nádoroszlop" (Palatine Column) and from bat guano soil gathered in the middle of "Vámpirok terme" (Vampire Hall).

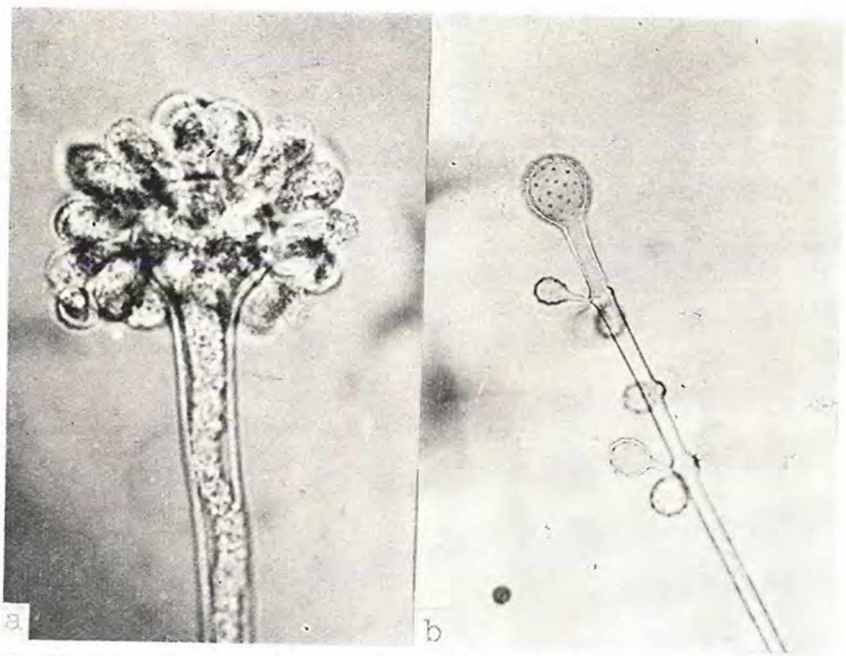

Fig. 10. *Cunninghamella elegans*. a: terminal head with rough conidia (800×), b: conidiophore (400×)

The colony is light grey, 20 – 25 mm high. The conidiophores have lateral branches, arranged singly, in pairs or a verticillate pattern. Here too the terminal vesicles are bigger than the lateral ones. The heads are 30 – 60 μ, conidia included, and 15 – 35 μ without conidia. There are two different types of rough conidia: spherical ones, 6 – 8 μ, and oval (droplike), 10 – 20 × 6 – 8 μ,

Summary

12 species belonging to the *Mucorales* series were isolated from soil samples and bat guano collected during the mycological examination of the stalactite cave "Baradla" at Aggtelek. These species were grown on different culture media

and identified as belonging to the genera *Mucor*, *Circinella*, *Actinomucor*, *Rhizopus*, *Absidia* and *Cunninghamella*.

In *Absidia glauca* the sexual cycle was observed and a + and a − mating type was separated from the initial culture.

For growing the strains the modified ToKaVa "bait" method was successfully applied.

Thanks are due to Mrs. Erzsébet Palik for her technical assistance.

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